IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
Before the Board of Patent Appeals and Interferences

In re Patent Application of Atty Dkt. 3929-3
C# M#

John W. CHERWONOGRODZKY TC/A.U.: 1645

Serial No. 09/866,801 Examiner: Ford

Filed: May 30, 2001 Date: February 28, 2006

Title: METHOD FOR DETECTING ANTIBODIES TO AND ANTIGENS OF FUNGAL AND YEAST EXPOSURES

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

☐ **Correspondence Address Indication Form Attached.**

☐ **NOTICE OF APPEAL**
Applicant hereby **appeals** to the Board of Patent Appeals and Interferences from the last decision of the Examiner twice/finally rejecting applicant's claim(s). $500.00 (1401)/$250.00 (2401) $

☒ An appeal **BRIEF** is attached in the pending appeal of the above-identified application $500.00 (1402)/$250.00 (2402) $ 500.00

☐ Credit for fees paid in prior appeal without decision on merits -$ ( )

☐ A reply brief is attached. **(no fee)**

☒ Petition is hereby made to extend the current due date so as to cover the filing date of this paper and attachment(s)
One Month Extension $120.00 (1251)/$60.00 (2251)
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Three Month Extensions $1020.00 (1253/$510.00 (2253)
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Less month extension previously paid on -$( )

TOTAL CREDIT CARD PAYMENT ENCLOSED $ 1520.00

Any future submission requiring an extension of time is hereby stated to include a petition for such time extension. The Commissioner is hereby authorized to charge any deficiency, or credit any overpayment, in the fee(s) filed, or asserted to be filed, or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our **Account No. 14-1140.** A duplicate copy of this sheet is attached.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Patent Application of
John W. CHERWONOGRODZKY — Atty. Ref.: 3929-3
Serial No. 09/866,801 — TC/A.U.: 1645
Filed: May 30, 2001 — Examiner: Ford
For: METHOD FOR DETECTING ANTIBODIES TO AND ANTIGENS OF FUNGAL AND YEAST EXPOSURES

* *

February 28, 2006

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APPEAL BRIEF

Sir:

Applicant hereby appeals the final rejection of claims 63-65 and 67-80, in the Office Action dated March 29, 2005, and submits the present Appeal Brief pursuant to 37 CFR § 41.37.

Table of Contents

(1) REAL PARTY IN INTEREST

The real party in interest is The Minister of National Defence of Government of Canada, National Defence Headquarter, 101 Colnel By Drive, Ottawa, Ontario, Canada KIA 0K2, by way of an Assignment from the appellants, submitted for recordation in the U.S. Patent and Trademark Office on October 17, 2001.

(2) RELATED APPEALS AND INTERFERENCES

The appellant, the appellant's legal representative, and the assignee are not aware of any related prior or pending appeals or interferences or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in this appeal.

(3) STATUS OF THE CLAIMS

Claims 63-65, 67-80, 83-86 and 88-90 are pending.

Claims 63-65 and 67-80 have been finally rejected. The remaining pending claims have been withdrawn from consideration.

The application was originally filed with 29 claims. Originally-filed claims 13-29 were canceled, and new claims 30-46 were added in an Amendment dated April 29, 2002. Claims 30, 43 and 46 were amended and claims 47-53 added by way of an Amendment dated October 18, 2002. Claims 54-62 were added in an Amendment dated August 25, 2003. Claims 1-62 were canceled and claims 63-91 added in an Amendment dated May 18, 2004. Claims 66, 81, 82, 87 and 91 were canceled in the Amendment After Final Rejection dated June 21, 2005.

The Amendment After Final Rejection dated June 21, 2005 was entered. See Advisory Action dated October 7, 2005, tick-box 7.

Claims 63-65 and 67-80 are the subject of the present appeal.

A copy of claims 63-65 and 67-80, i.e., the claims involved in the appeal, is attached as a Claims Appendix, pursuant to Rule 41.37(c)(1)(viii).

(4) STATUS OF THE AMENDMENTS

An Amendment was on June 21, 2005 in response to the final Office Action dated March 29, 2005. The amendment After Final Rejection has been entered for the purposes of Appeal. See Advisory Action dated October 7, 2005, tick-box 7.

(5) SUMMARY OF CLAIMED SUBJECT MATTER

The presently claimed invention provides an antigenic composition for detecting anti-aflatoxin antibodies from a sample of a test subject. The composition of the claimed invention contains a fungal or yeast cell culture supernatant containing fungal or yeast components shed into the supernatant during culturing; wherein the antigenic composition is characterized by a reduction of antigenic activity of less than 20%, as measured by ELISA, after treatment with protease in 0.25M TRIS buffer at pH 7.2. See independent claim 63, and page 5, 4th full paragraph, and page 16, last paragraph, for example, of the specification.

The present application provides, for the first time, an antigenic composition which includes fungal or yeast cell components which are substantially non-proteinaceous (i.e., the antigenic source is characterized by a reduction of antigenic activity of less than 20%, as measured by ELISA, after treatment with protease in 0.25 M TRIS buffer at pH 7.2).

The composition of the claimed invention is further separately characterized as being obtained from a supernatant containing a mixture of antigens which are capable of binding to different fungal or yeast species (see dependent claim 64 and page Table V on page 25 of the application and accompanying text), as containing fungal or yeast aflatoxin (see dependent claim 65 and page 16, last paragraph as well as the originally-field claims), and as containing components capable of binding said

antibodies (see dependent claim 67 and page 17, second full paragraph and Table VI of the specification).

The composition of the claims is further characterized as being prepared from a supernatant prepared and used at a temperature above the freezing point of the composition. See dependent claim 68 and the specification at page 17, first paragraph and originally-filed claim 14.

The composition of the claims is further characterized as being obtained from a supernatant prepared and used at a temperature of 20 ^{o}C or at a temperature above the freezing point of the composition and under aeration conditions or at a temperature above the freezing point of the composition and under aeration conditions of gentle shaking. See dependent claims 69-71 and the specification at page 8, first full paragraph and originally-filed claim 15, page 16, first paragraph and originally-filed claim 16, and originally-filed claim 17.

The details of dependent claim 72 find support, for example, at page 18 and Tables VI-VIII.

The details of dependent claim 73 find support, for example, at page 18, last paragraph and Table VII.

The details of dependent claim 74 find support, for example, at page 18, last paragraph and Table VIII.

The details of dependent claim 75 find support, for example, at page 19, first paragraph and Table VIII.

The details of dependent claim 76 find support, for example, at page 19, first paragraph and the Tables as well as originally-filed claim 26.

The details of independent claim 77 find support, for example, at page 19, and Table IX as well as originally-filed claim 27.

The details of dependent claims 78 and 79 find support, for example, at page 5, penultimate paragraph, 17, 2nd paragraph and originally-filed claim 28.

The details of dependent claim 80 find support, for example, at page 18, last paragraph and Table VII and originally-filed claim 19.

As further explained below, the cited art fails to teach or suggest the presently claimed invention.

(6) GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The following grounds of rejection is presented for review:

(1) Whether the invention of claims 63-65, 67-73, 75-76 and 78-79 is anticipated under 35 U.S.C. § 102 by Pasarell (Journal of Clinical Microbiology, July 1990, pp 1665-1657);

(2) Whether the invention of claims 63-65, 67-68, 73 and 78-80 is anticipated under 35 U.S.C. § 102 by Dubeau (Biotechnology Letters, 1987, Vol. 9, No. 4, pp 275-280 Abstract only);

(3) Whether the invention of claims 63-64, 67, 70-72, and 77-78 is anticipated under 35 U.S.C. § 102 by Honbo (Sabouraudia: Journal of Medical and Veterinary Mycology, 1984, 22, 301-310); and

(4) Whether the invention of claims 63-68, 72, 74, 76 and 78-79 is anticipated under 35 U.S.C. § 102 by Groopman (U.S. Patent No. 4,859,611).

(7) ARGUMENT

The invention of claims 63-65 and 67-80 is patentable over the cited art. Consideration of the following in this regard is requested.

The above-identified application provides, for the first time, an antigenic composition which includes fungal or yeast cell components which are substantially non-proteinaceous (i.e., the antigenic source is characterized by a reduction of antigenic activity of less than 20%, as measured by ELISA, after treatment with protease in 0.25 M TRIS buffer at pH 7.2). As has been explained to the Examiner in a personal interview of April 28, 2004, antigens of the cited art are described as being proteinaceous, as further detailed below. Moreover, one of ordinary skill in the art will appreciate that proteinaceous antigens will be rendered substantially non-antigenic by treatment with protease. The presently claimed invention however is characterized as only having a minimal loss in antigenic activity when treated with protease.

Further, one of ordinary skill in the art will appreciate that proteinaceous antigens may generally be frozen or lyophilized without substantial loss of antigenicity. The invention of dependent claims 68-71 require preparation and use above freezing in view of the applicants disclosure at page 17, first paragraph, for example, wherein a substantial reduction of antigenic activity of compositions of the invention resulted from freezing.

In characterizing the antigen-containing compositions of present invention, the applicants have described the physical properties of the claimed compositions as

being generally resistant to proteases (i.e., reduction of antigenic activity of less than 20%, as measured by ELISA, after treatment with protease in 0.25 M TRIS at pH 7.2 (see, page 16, last paragraph of the specification, for example) and sensitive to freezing (see, page 8, last paragraph of the specification, for example).

Independent claim 63, for example, includes a characterization of the physical properties of the antigens of the invention by reference to a test method, i.e., ELISA after treatment with protease under the given conditions. The recitation of a reference to a method in independent claim 63 does not make these claims product-by-process (i.e., products defined by their method of manufacture) but rather the recitation of the reference method is similar to the physical characterization of the molecular weight of a protein by reference to measurement by a method of electrophoresis, such as by an SDS-PAGE technique.

(1) Claims 63-65, 67-73, 75-76 and 78-79 are patentable over Pasarell (Journal of Clinical Microbiology, July 1990, pp 1665-1657) and reversal of the Section 102 rejection of claims 63-65, 67-73, 75-76 and 78-79 based on the same is requested.

Pasarell fails to teach each and every aspect of the presently claimed invention.

Initially, it is noted for completeness that the requirement of independent claim 63 for detection of "anti-aflatoxin" antibodies was the subject of now-

canceled dependent claim 66 which was indicated as being patentable over Pasarell in the Office Action of March 29, 2005 (i.e., claims not rejected as assumed patentable). See pages 3-5 of the Office Action. The Section 102 rejection of independent claim 63 and all of the presently-rejected dependent claims 65, 67-73, 75-76 and 78-79, over Pasarell is therefore a new rejection not necessitated by the applicants Amendment After Final Rejection of June 21, 2005. The fact that these claim may have been rejected over other art in the Final Office Action does not negative the fact that claim 66 was not rejected over Pasarell. See the Examiner's comment on page 4 of the Advisory Action Attachment dated October 7, 2005. The Amendment After Final Rejection dated June 21, 2005 should have overcome the Section 102 rejection of the noted claims over Pasarell. As the Amendment has been entered, any rejection over Pasarell should have been presented in a new, non-final Office Action.

Substantively, the pending claims are patentable over Pasarell as, for example, Pasarell prepared their antigen composition by concentration with a PM10 filter. Such a procedure will be recognized by one of ordinary skill in the art to have discarded any material in the culture filtrate which was less than 10,0000 m.w., while retaining larger material, such as antigenic proteins. Pasarell does not literally identify the chemical nature of the antigenic material. One of ordinary skill in the present art, at the time Pasarell was published, would have expected that the

antigenic material of Pasarell's filtrate was larger (i.e., > 10,000 m.w.) proteins, which is why the concentration of the antigen-containing filtrate with a PM10 filter was not expected to alter the antigenic character of the filtrate.

As Pasarell fails to literally or inherently teach an antigenic composition characterized by a reduction of antigenic activity of less than 20%, as measured by ELISA, after treatment with protease in 0.25 M TRIS buffer at pH 7.2 (i.e., an antigenic composition which is substantially not proteinaceous), the invention of, for example, claim 63 is submitted to be patentable over Pasarell.

Recitation in claim 63 of the use of the composition for detecting anti-aflatoxin antibodies, which was the subject of now-canceled claim 66, emphasizes this distinction over the cited art and is likely a reason the Examiner previously indicated that now-canceled claim 66 was patentable over Pasarell.

The invention of, for example, claim 65, is similarly patentable over Pasarell. As evidenced by, for example, Brewer et al (Can J. Microbiol. 24:1082-1086 (1978) (copy attached as evidence exhibit (a)) fungal and yeast toxins, such as aflatoxins, are expected to be non-proteinaceous and of a molecular weight which would be excluded from the antigenic composition of Pasarell by, for example, the PM10 filter used by Pasarell in preparation of their antigenic composition.

For completeness, the applicant notes the Examiner's comment at page 5 of the Office Action dated November 18, 2003 (Paper No. 16) and repeated in the Final Office Action of March 29, 2005 (page 5) and incorporated by reference in the rejection maintained in the Advisory Action of October 7, 2005, that

"There is no requirement in the claims that requires that the cell culture supernatant be filtered with a specific filter."

The Examiner's comment however is not understood, and clarification has been previously requested by the applicant at page 9 of the Amendment dated May 18, 2004, as it is the cited art (i.e., Pasarell) which requires a specific filter and it is the presently claimed invention which rather does not require a specific filtration step.

The invention of claim 63, for example, provides a cell culture supernatant containing fungal or yeast components shed into the supernatant during culturing. The filtration step of Pasarell has removed many of the fungal or yeast components shed into the supernatant during culturing.

(7)(a)(1) ARGUMENT pursuant to 37 CFR § 41.67(vii), ¶2, 2nd sentence - rejection of claims 68-71 argued separately

Claims 68-71 should be considered separately with regard to the Section 102 rejection over Pasarell as the cited document fails to literally or inherently teach the temperature of preparation of the claimed composition required by these claims.

The Examiner 's states that the process of preparation of the claimed composition in, for example, claims 68-71 is a "matter of design choice." See page 2 *et seq.* of the Attachment to the Advisory Action. Whether a matter of design choice or not, the applicant notes that the fact that Pasarell fails to literally or inherently teach the recited claim requirement is believed to be an acknowledgement of novelty of the claims over the cited art as the cited art fails to teach each and every aspect of

the claimed invention. Moreover, the Examiner does not need "facilities for examining and comparing applicant's fungal or yeast culture supernatant with the fungal or yeast culture supernatant of the prior art..." (see page 3 of the Attachment to the Advisory Action) as the applicants has demonstrated in the present specification the advantage of the recited temperature of preparation and use thereof in providing a superior and structurally/functionally unique product.

(7)(b)(1) ARGUMENT pursuant to 37 CFR § 41.67(vii), ¶2, 2nd sentence - rejection of claim 64 argued separately

Claim 64 should be considered separately with regard to the Section 102 rejection over Pasarell as the cited document fails to literally or inherently teach the cross-reactivity of the claimed invention. In fact, the applicant submits that Table 1 on page 1656 of the cited art fails to teach cross-reactivity of the components of the cited art.

(7)(c)(1) ARGUMENT pursuant to 37 CFR § 41.67(vii), ¶2, 2nd sentence - rejection of claim 65 argued separately

Claim 65 should be considered separately with regard to the Section 102 rejection over the cited Pasarell as the cited document fails to literally or inherently teach a supernatant of the claim which contains aflatoxin. The evidence of record clearly establishes that aflatoxin of culture supernatants, if they existed, where often separated and discarded by filtration and/or destroyed by process conditions. As the

cited abstract further fails to teach or suggest the presence of aflatoxin, claim 65 is further submitted to be novel over the cited art.

(7)(d)(1) ARGUMENT pursuant to 37 CFR § 41.67(vii), ¶2, 2nd sentence - rejection of claim 69 argued separately

Claim 69 should be considered separately with regard to the Section 102 rejection over the cited Pasarell as the cited document fails to literally or inherently teach a preparation of the claimed supernatant composition of the claim which is prepared and used at 20°C. Rather, the cultures of the cited art are prepared at a temperature of 25°C or greater, filtered (as described above) and used at a temperature of 25°C. See pages 1655-6 of Pasarell. As the cited reference fails to teach the requirement of claim 69, claim 69 should be considered separately novel over the cited art.

The claims are submitted to be patentable over Pasarell and reversal of the Section 102 rejection of claims 63-65, 67-73, 75-76 and 78-79 over Pasarell is requested.

(2) Claims 63-65, 67-68, 73 and 78-80 are patentable over Dubeau (Biotechnology Letters, 1987, Vol. 9, No. 4, pp 275-280 abstract only) and reversal of the Section 102 rejection of claims 63-65, 67-68, 73 and 78-80

based on the same is requested. (Previously-rejected claim 81 was canceled in the Amendment of June 21, 2005.)

The cited Dubeau abstract fails to teach each and every aspect of the presently claimed invention.

Initially, it is noted for completeness that the requirement of independent claim 63 for detection of "anti-aflatoxin" antibodies was the subject of now-canceled dependent claim 66 which was indicated as being patentable over Dubeau in the Office Action of March 29, 2005 (i.e., claims not rejected as assumed patentable). See pages 9-11 of the Office Action. The Section 102 rejection of independent claim 63 and all of the presently-rejected dependent claims 64-65, 67-68, 73 and 78-80, over Dubeau is therefore a new rejection not necessitated by the applicants Amendment After Final Rejection of June 21, 2005. The fact that these claim may have been rejected over other art in the Final Office Action does not negative the fact that claim 66 was not rejected over Dubeau. See the Examiner's comment on page 8 of the Advisory Action Attachment dated October 7, 2005. The Amendment After Final Rejection dated June 21, 2005 should have overcome the Section 102 rejection of the noted claims over Dubeau. As the Amendment has been entered, any rejection over Dubeau should have been presented in a new, non-final Office Action.

Substantively, the pending claims are patentable over Dubeau for any of the following reasons.

The following is the total disclosure of Dubeau relied upon by the Examiner:

> Maximum xylanase production by **Chaetomium** cellulolyticum was obtained in the **culture supernatant** after 30 h of growth at 37° C in basal medium containing 1% xylan at pH maintained between 6.5 and 7.5. Addition of 0.05% Tween 80 to the medium increased the enzyme production considerably. Xylanase production was found to be growth associated. The optimal conditions for enzymatic hydrolysis of xylan were found to be pH 6.0 and 50° C. During enzymatic hydrolysis, xylose, xylobiose and other xylooligosaccharides were liberated from xylan. The pH values for xylanase production and for xylan hydrolysis were closely related to the utilization of hemicelluloses of aspen wood for fungal protein production by this organism as reported in our earlier work.

A complete copy of the reference has not been provided or relied upon by the Examiner. A complete copy of the reference was not previously requested by the applicant as the inclusion of the details of now-canceled dependent (and previously indicated as patentable) claim 66 in to independent claim 63 appeared to not require a more detailed review of the cited reference. Claim 63 was previously amended to advance prosecution by reducing the issues for appeal.

The cited abstract does not literally or inherently teach an antigenic composition or the present claims. The cited abstract provides a culture supernatant without any specific indication of how the supernatant was prepared such that it is uncertain whether the supernatant is an antigenic composition of the present claims. This uncertainty suggests that the

presently claimed invention is novel over the cited abstract as the claimed invention does not necessarily flow from the teaching of the cited abstract.

The cited abstract does not literally or inherently teach an antigenic composition of the present claims which comprises a mixture of antigens which are capable of binding to different fungal or yeast species.

The cited abstract does not literally or inherently teach an antigenic composition of the present claims which comprises fungal or yeast aflatoxin.

The cited abstract does not literally or inherently teach an antigenic composition of the present claims which contains components capable of binding anti-aflatoxin antibodies.

The cited abstract does not literally or inherently teach use of the composition of the present claims at a temperature above the freezing point of the composition.

The cited abstract does not literally or inherently teach an antigenic composition of the present claims in the form of a vaccine.

The Examiner's suggestion that any culture supernatant of *Chaetomium* would allegedly anticipate the present claims 63-65, 67-68, 73 and 78-80 is, to the extent the applicant understand this to be the Examiner's position, inappropriate and scientifically incorrect as the applicant has demonstrated, for

example, that structurally and functionally distinct products are obtained based on preparation and storage conditions.

(7)(a)(2) ARGUMENT pursuant to 37 CFR § 41.67(vii), ¶2, 2nd sentence - rejection of claims 63-65, 67-68, 78 and 79 argued separately

Claims 63-65, 67-68, 78 and 79 should be considered separately with regard to the Section 102 rejection over the cited Dubeau abstract as the cited document fails to literally or inherently teach a supernatant of the claims, or any supernatant other than reference to the highlighted "xylanase production by Chaetomium cellulolyticum ... obtained in the culture supernatant". As claims 63-65, 67-68, 78 and 79 do not specifically require or refer to *Chaetomium*, the claims are further submitted to be novel over the cited abstract.

(7)(b)(2) ARGUMENT pursuant to 37 CFR § 41.67(vii), ¶2, 2nd sentence - rejection of claim 65 argued separately

Claim 65 should be considered separately with regard to the Section 102 rejection over the cited Dubeau abstract as the cited document fails to literally or inherently teach a supernatant of the claim which contains aflatoxin. The evidence of record clearly establishes that aflatoxin of culture supernatants, if they existed, where often separated and discarded by filtration and/or destroyed by process conditions.

As the cited abstract further fails to teach or suggest the presence of aflatoxin, claim 65 is further submitted to be novel over the cited abstract.

The claims are submitted to be patentable over the Dubeau abstract and reversal of the Section 102 rejection of claims 63-65, 67-68, 73 and 78-80 over the Dubeau abstract is requested.

(3) Claims 63-64, 67, 70-72, and 77-78 are patentable over Honbo (Sabouraudia: Journal of Medical and Veterinary Mycology, 1984, 22, 301-310) and reversal of the Section 102 rejection of claims 63-64, 67, 70-72, and 77-78 (claim 82 having been canceled in the Amendment of June 21, 2005) based on the same is requested.

The cited Honbo fails to teach each and every aspect of the presently claimed invention.

Initially, it is noted for completeness that the requirement of independent claim 63 for detection of "anti-aflatoxin" antibodies was the subject of now-canceled dependent claim 66 which was indicated as being patentable over Honbo in the Office Action of March 29, 2005 (i.e., claims not rejected are assumed patentable). See pages 11-12 of the Office Action. The Section 102 rejection of independent claim 63 and dependent claims 64, 67, 70-72 and 78,

over Honbo is therefore a new rejection not necessitated by the applicants Amendment After Final Rejection of June 21, 2005. The fact that these claim may have been rejected over other art in the Final Office Action does not negative the fact that claim 66 was not rejected over Honbo. See the Examiner's comment on page 9 of the Advisory Action Attachment dated October 7, 2005. The Amendment After Final Rejection dated June 21, 2005 should have overcome the Section 102 rejection of the noted claims over Honbo. As the Amendment has been entered, any rejection over Honbo should have been presented in a new, non-final Office Action.

Substantively, the pending claims are patentable over Honbo as, for example, Honbo prepared their antigen composition by concentration with a PM10 filter. See page 302, paragraph titled "Antigen production" of Honbo. Such a procedure will be recognized by one of ordinary skill in the art to have discarded any material in the culture filtrate which was less than 10,0000 m.w., while retaining larger material, such as antigenic proteins. Honbo does not literally identify the chemical nature of the antigenic material. One of ordinary skill in the present art, at the time Honbo was published, would have expected that the antigenic material of Honbo's filtrate was larger (i.e., > 10,000 m.w.) proteins, which is why the concentration of the antigen-containing filtrate with a PM10 filter was not expected to alter the antigenic character of the filtrate.

As Honbo fails to literally or inherently teach an antigenic composition characterized by a reduction of antigenic activity of less than 20%, as measured by ELISA, after treatment with protease in 0.25 M TRIS buffer at pH 7.2 (i.e., an antigenic composition which is substantially not proteinaceous), the invention of, for example, independent claims 63 and 77, as well as claims dependent therefrom, are submitted to be patentable over Honbo.

Recitation in claim 63 of the use of the composition for detecting anti-aflatoxin antibodies, which was the subject of now-canceled claim 66, and the recitation in claim 77 of the presence of aflatoxin, emphasize this distinction over the cited art and is likely a reason the Examiner previously indicated that now-canceled claim 66 was patentable over Honbo.

The invention of, for example, dependent claim 65 and independent claim 77, are similarly patentable over Honbo. As evidenced by, for example, Brewer et al (Can J. Microbiol. 24:1082-1086 (1978) (copy attached as evidence exhibit (a)) fungal and yeast toxins, such as aflatoxins, are expected to be non-proteinaceous and of a molecular weight which would be excluded from the antigenic composition of Honbo by, for example, the PM10 filter used by Honbo in preparation of their antigenic composition.

The claimed compositions would be recognized by one of ordinary skill in the art to be structurally and functionally distinct from the antigenic compositions of Honbo.

(7)(a)(3) ARGUMENT pursuant to 37 CFR § 41.67(vii), ¶2, 2nd sentence - rejection of claim 65 argued separately

Claim 65 should be considered separately with regard to the Section 102 rejection over the cited Honbo as the cited document fails to literally or inherently teach a supernatant of the claim which contains aflatoxin. The evidence of record clearly establishes that aflatoxin of culture supernatants, if they existed, where often separated and discarded by filtration and/or destroyed by process conditions. As the cited abstract further fails to teach or suggest the presence of aflatoxin, claim 65 is further submitted to be novel over the cited art.

(7)(b)(3) ARGUMENT pursuant to 37 CFR § 41.67(vii), ¶2, 2nd sentence - rejection of claim 77 argued separately

Claim 77 should be considered separately with regard to the Section 102 rejection over the cited Honbo as the cited document fails to literally or inherently teach a supernatant of the claim which contains aflatoxin shed during the culturing of *Cladosporium*. The evidence of record clearly establishes that aflatoxin of culture supernatants, if they existed, where often separated and discarded by filtration and/or destroyed by process conditions. As the cited abstract further fails to teach or suggest the presence of aflatoxin, claim 77 is further submitted to be novel over the cited art.

The claims are submitted to be patentable over the Honbo and reversal of the Section 102 rejection of claims 63-65, 67, 73 and 78-80 over the Dubeau abstract is requested.

(4) Claims 63-65, 67-68, 72, 74, 76 and 78-79 are patentable over Groopman (U.S. Patent No. 4,859,611) and reversal of the Section 102 rejection of claims 63-65, 67-68, 72, 74, 76 and 78-79 based on the same is requested.

The cited Groopman fails to teach each and every aspect of the presently claimed invention.

Specifically, Groopman teaches production of antibodies which bind to aflatoxin. Groopman does not teach or provide an antigenic composition containing a cell culture supernatant according to the presently claimed invention.

Groopman describes conjugation of aflatoxin B_1 (AFB_1) to bovine gamma globulin (BGG) for preparation of an immunogen. See, column 4, lines 25-26 of Groopman. One of ordinary skill in the art will appreciate that gamma globulin is a proteinaceous material. See Dorland's Illustrated Medical Dictionary, such as available on-line at http://www.mercksource.com:

> globulin (glob·u·lin) (glob′u-lin) [L. globulus globule] any member of a class of proteins, most of which are insoluble in water but soluble in saline solutions (euglobulins), but some of which (pseudoglobulins) are water soluble proteins whose other physical properties closely resemble those of the true globulins. See also serum g's.

> γ-g.'s , gamma g.s serum globulins having the least rapid electrophoretic migration. Since the gamma globulin fraction is composed almost entirely of immunoglobulin, gamma globulin came to be used as a synonym of "immunoglobulin" or "immune globulin." Because some immunoglobulins have α or β electrophoretic mobility, this usage is imprecise and is in decline.

As the material of the cited art is proteinaceous, one of ordinary skill in the art would expect that the antigenicity of the composition of the cited art would be markedly reduced by treatment with protease. The cited art therefore does not teach each and every aspect of the presently claimed invention.

The source of the AFB_1 is not described in Groopman. Groopman does not teach (literally or inherently) or suggest that the AFB_1 of the immunogen preparation method of Groopman is presented, used or prepared in a composition according to the presently claimed invention.

Groopman describes the use of a radio-labelled ^3H-aflatoxin B_1 as a tracer in the design of a detection method. See, Example 1 of Groopman. A method for preparing the tracer is not provided by Groopman. Groopman does not teach (literally or inherently) or suggest that the radio-labelled ^3H-aflatoxin B_1 tracer preparation method of Groopman is presented, used or prepared in a composition according to the presently claimed invention.

Groopman describes, prophetically, the isolation and detection of aflatoxin in vitro from human urine, serum and milk samples. See, column 11, lines 15-21 of Groopman. These compositions of Groopman, to the extent the

prophetic descriptions may be considered a teaching in the art, are not compositions according to the presently claimed invention.

Groopman fails to teach the presently claimed composition comprising a fungal or yeast cell culture supernatant.

(7)(a)(4) ARGUMENT pursuant to 37 CFR § 41.67(vii), ¶2, 2nd sentence - rejection of claim 74 argued separately

Claim 74 should be considered separately with regard to the Section 102 rejection over the cited Groopman as the cited document fails to literally or inherently teach a supernatant of the claim which contains a mixture of antigens which are capable of binding *Aspergillus* and *Paecilomyces*. The cited patent is not believed to provide for reactivity of any specific combination of different fungal or yeast species. As the cited abstract further fails to teach or suggest the presence of components required by claim 74, the claim is further submitted to be novel over the cited art.

The claims are submitted to be patentable over the Groopman and reversal of the Section 102 rejection of claims 63-65, 67-68, 72, 74, 76 and 78-79 over the Groopman is requested.

The claims are submitted to be in condition for allowance and Reversal of the Final Rejections is requested.

Respectfully submitted,

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(8) CLAIMS APPENDIX

63. An antigenic composition for detecting anti-aflatoxin antibodies from a sample of a test subject, said composition comprising a fungal or yeast cell culture supernatant containing fungal or yeast components shed into the supernatant during culturing; said antigenic composition being characterized by a reduction of antigenic activity of less than 20%, as measured by ELISA, after treatment with protease in 0.25M TRIS buffer at pH 7.2.

64. The composition of claim 63 wherein said supernatant comprises a mixture of antigens which are capable of binding to different fungal or yeast species.

65. The composition of claim 63 wherein said supernatant comprises fungal or yeast aflatoxin.

67. The composition of claim 63 wherein said components are capable of binding said antibodies.

68. The composition of claim 63, wherein said supernatant is prepared and used at a temperature above the freezing point of said composition.

69. The composition of claim 68, wherein said supernatant is prepared and used at 20 ^{o}C .

70. The composition of claim 68, wherein said supernatant is prepared under aeration condition.

71. The composition of claim 70, wherein said aeration condition is provided by gentle shaking.

72. The composition of claim 63, wherein said supernatant displays specific antibody affinity such that only antibodies of a specific fungus or yeast bind to said components.

73. The composition of claim 63, wherein said fungal or yeast cells are selected from species selected from the group of *Alternaria, Baker's Yeast, Chaetomium* and *Fusarium*.

74. The composition of claim 64, wherein said different fungal or yeast species are selected from *Aspergillus* and *Paecilomyces*.

75. The composition of claim 63, wherein said supernatant is from a cell culture of species selected from the group of *Bipolaris, Neosatorya, Penicillium, Stachybotrys* and *Uliocladium.*

76. The composition of claim 63 wherein said supernatant is from a fungal cell culture supernatant of *Biopolaris*.

77. An antigenic composition comprising a fungal cell culture supernatant of *Cladosporium* comprising aflatoxin shed into the supernatant during culturing said fungal cell culture.

78. A vaccine comprising a composition of claim 63.

79. A vaccine comprising a composition of claim 65.

80. The composition of claim 63 wherein said supernatant is from cell culture of *Chaetomium.*

(9) EVIDENCE APPENDIX

Attached:

(a) Brewer et al (Can J. Microbiol. 24:1082-1086 (1978))

Cited by the Examiner and listed on the PTO 892 which is "Part of Paper No. 16"

(9) RELATED PROCEEDINGS APPENDIX

Attached:

NONE

The production of toxic metabolites by *Chaetomium* spp. isolated from soils of permanent pasture[1]

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Accepted June 7, 1978

BREWER, D., and A. TAYLOR. 1978. The production of toxic metabolites by *Chaetomium* spp. isolated from soils of permanent pasture. Can. J. Microbiol. 24: 1082–1086.

One hundred and two isolates of *Chaetomium* spp. have been identified from 2563 soil samples collected from permanent pasture at Nappan, Nova Scotia. *Chaetomium umbonatum* was the *Chaetomium* species most commonly isolated. Fifty-six of the *Chaetomium* isolates were grown in the laboratory and the cultures examined for the production of toxic metabolites. The culture filtrates of 12, and extracts of mycelium of 18, of these isolates inhibited bacterial growth. Chetomin was detected in nine mycelium extracts and isolated from four of the mycelium extracts. Chaetoglobosins were isolated from three mycelium extracts.

BREWER, D., et A. TAYLOR. 1978. The production of toxic metabolites by *Chaetomium* spp. isolated from soils of permanent pasture. Can. J. Microbiol. 24: 1082–1086.

Nous avons isolé et identifié 102 isolats du genre *Chaetomium* à partir de 2563 échantillons de sols collectionnés à Nappan, en Nouvelle Ecosse, sur des pâturages permanents. *Chaetomium umbonatum* a été de l'espèce *Chaetomium* le plus communément isolée. Nous avons fait pousser au laboratoire 56 isolats de *Chaetomium* et nous en avons examiné la production de métabolites toxiques. Les filtrats de culture de 12 isolats et les extraits de mycélium de 18 isolats ont inhibé la croissance bactérienne. La "chetomin" a été détectée dans neuf extraits de mycélium, et isolée dans quatre. Les "chaetoglobosins" ont ete isolées dans trois des extraits de mycélium.

[Traduit par le journal]

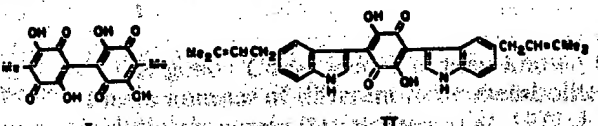
Introduction

The genus *Chaetomium* is now known to produce a number of different toxic metabolites; they include oösporein (I), (Brewer *et al.* 1977; Lloyd *et al.* 1955); cochliodinol (II), (Brewer *et al.* 1970); chaetocin (III), (Hauser *et al.* 1970); colletodiol (IV), (Grove *et al.* 1966); chetomin (V), (Safe and Taylor 1972); and the chaetoglobosins (VI), Sekita *et al.* 1973, 1976). The chetomins and chaetoglobosins have been implicated in mycotoxicoses (Brewer, Duncan, *et al.* 1972; Saito *et al.* 1971). We have therefore examined our collection of this genus, isolated from samples of soil taken during the past 10 years from permanent pastures in Nova Scotia, to determine the frequency of *Chaetomium* isolates producing toxic metabolites.

Materials and Methods

Organisms

Soil samples were collected from the experimental areas P2 (upland) and R3 (marshland) at Agriculture Canada, Experimental Farm, Nappan, Nova Scotia. The pastures have been described in detail (Brewer *et al.* 1971). The *Chaetomium* isolates were obtained from soil plugs by the dilution method (Brewer *et al.* 1971) except for one isolate of *C. cochliodes* which was isolated from the surface of a root (Brewer *et al.* 1971). Among the isolates five species were identified: *C. cochliodes* Palliser, *C. globosum* Kunze, *C. umbonatum* Brewer (Brewer 1973), *C. spinosum* Chivers, and *C. funicolum* Cooke.



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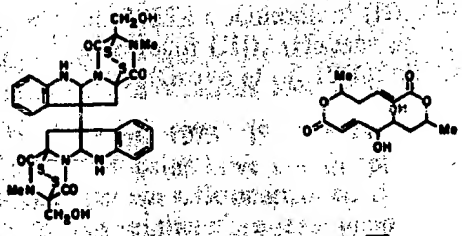

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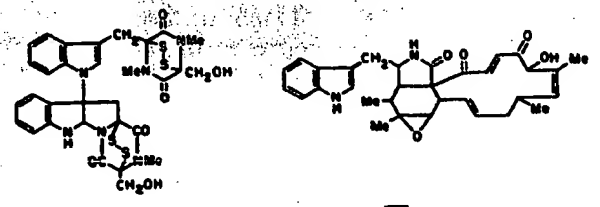

V VI

Bacillus subtilis (Ehrenberg) Cohn (HLX 373[2]) was used as the test organism to determine growth inhibition (Brewer *et al.* 1966).

Cultivation of Chaetomium Isolates

Vegetative inocula were prepared as described (Brewer,

[1]NRCC No. 16772.

[2]Accession number to the culture collection held at this laboratory.

Duncan, *et al.* 1972) and the medium used for the growth of the isolates for metabolite production was the basal medium (Brewer, Duncan, *et al.* 1972) supplemented with corn steep liquor (5 ml/ℓ). Portions of the supplemented medium (200 ml) in Erlenmeyer flasks of capacity 500 ml were inoculated with the vegetative inoculum (1 ml) and incubated at 25°C on a rotatory shaker (Brewer, Duncan, *et al.* 1972).

Isolation and Extraction of Chaetomium Metabolites

The cultures (two flasks per isolate) were filtered, the mycelia macerated with ice (30 g) and water (100 ml), and the slurry lyophilized. The dry mycelium (1.9–6.3 g) was macerated with benzene (50 ml), filtered, and the extraction repeated 3 times. The combined benzenoid extracts were evaporated; the residue (14–113 mg) dissolved in methanol (10 ml) and petroleum ether (10 ml), water (0.5 ml) added, and the phases separated. The methanolic phase was extracted 3 times with petroleum ether and then evaporated. Hereinafter the residue (5–25 mg) is called the extract. Chetomin was estimated by an antibacterial test (see below) or by titration (Brewer and Taylor 1967).

On the scale used for the isolation of toxic metabolites, the supplemented medium (1 ℓ) in Erlenmeyer flasks of capacity 2 ℓ were inoculated with 4 ml of the inoculum. The chetomin fermentations were cultivated and harvested and the antibiotic was isolated as described (Safe and Taylor 1972). The chaetoglobosin fermentations were grown on the 20-ℓ scale at 25°C for 7 days and the toxins isolated as follows. The fungal tissue was macerated, lyophilized (about 220 g), and extracted with benzene and the extract partitioned between methanol – petroleum ether – water (9.9:10:0.1) as described for the isolation of chetomin (Safe and Taylor 1972). The residue from the methanolic raffinate (1.96 g) was chromatographed on silica gel (Merck) plates (100 × 20 × 0.1 cm), using ethyl acetate – benzene (9:11) as the developing solvent. Two yellow bands of R_f 0.45 and 0.32 were scraped off the plate, eluted from the silica with ethyl acetate, the solution was evaporated, and in the case of the metabolite of R_f 0.32, the residue was recrystallized from toluene.

Antibacterial Tests

Culture filtrates (5 ml) were diluted with the growth medium (× 2 concentration, 5 ml) (Brewer *et al.* 1966) and a duplicated series of 10-fold dilutions of this solution were inoculated with about 10^7 cells of *B. subtilis*. The cultures were incubated at 37°C for 18 h when their optical densities at 650 nm were determined, and the organisms examined for motility, microscopically. The mycelium extracts (10 mg) were dissolved in ethanol (1 ml) and the solution (0.1 ml) was diluted with the growth medium (9.9 ml, Brewer *et al.* 1966) which was inoculated and incubated. The presence or absence of growth was determined as described for the culture-filtrate tests. Controls containing water (1 ml) or ethanol (1 ml) or chetomin (0.1 μg/ml) or chaetoglobosin (1 mg/ml) were always inoculated and incubated with the test cultures.

Thin-layer Chromatography

Silica gel (Merck) plates (20 × 20 × 0.05 cm) containing a fluorescent indicator (λ_e 254 nm) were used for the chromatography of mycelium extracts. The extract (10 mg) in ethanol (1 ml) was applied (5 μl) to the plate and the chromatogram developed with chloroform – acetic acid (19:1). Metabolites were detected by irradiation with short-wave ultraviolet light (λ < 300 nm) and also by spraying the plates with silver nitrate solution (Rahman *et al.* 1970).

Physical Measurements

Infrared spectra were determined on samples dispersed in potassium bromide; spectra were obtained with a Perkin–Elmer 237 spectrometer. Mass spectra were obtained using a Dupont 21-110b mass spectrometer; ions were detected electrically and photographically; precise mass measurements were made by the peak-matching method using a suitable ion in the spectrum of perfluorokerosene as a standard. Ultraviolet spectra were measured on a Beckman DK-2 instrument.

Results and Discussion

We have attempted to assess the relative abundance of *Chaetomium* spp. in the fungal flora of permanent pasture at Nappan, Nova Scotia, in Table 1. The mean number of propagules per gram of upland soil was about 7×10^5 (Brewer *et al.* 1971); when *Chaetomium* spp. were isolated, the numbers of propagules per gram of this genus lay in the range 2×10^3 and 9×10^4. In a particular soil sample *Chaetomium* spp. accounted for 0.2–25% of the propagules grown in the laboratory. In marshland soil samples (which supported fewer fungal propagules that could be cultivated, 4×10^5/g, Brewer *et al.* 1971), the number of propagules of *Chaetomium* spp. isolated varied between 0.9×10^3 and 4×10^5/g, and in individual soil samples accounted for 1–35% of the fungal propagules cultivated. It follows that the *Chaetomium* spp. obtained in culture (Table 1) represent a small proportion of the total isolated (though not necessarily in the field) and, hence they escaped selection in our screen of a random sample (Brewer, Taylor, and Hoehn 1972). *Chaetomium umbonatum* was the species most frequently isolated (Table 1) and appeared to be more common in upland soil samples. Some reservations concerning the latter conclusion must be made because more soil samples were collected from the upland soil in those years when both soils were investigated and sometimes very large numbers (up to 4×10^5/g of soil) of propagules of *C. umbonatum* were cultivated from marshland soil samples. Some *Chaetomium* isolates take several years to form perithecia in laboratory culture. Hence the smaller values in Table 1 in recent years may be explained.

Species other than *C. umbonatum* that were isolated included one isolate of *C. spinosum* from a marshland soil sample collected in 1967; seven isolates of *C. globosum*, two from upland soil samples collected in 1966 and 1975, and five from marshland soil samples collected in 1966 (1), 1967 (3), and 1968 (1); three isolates of *C. cochliodes* were obtained, two from the upland soil (1966 and 1967) and the other from the marshland soil (1966). Two distinct cultural strains of *C. funicolum* have been isolated. The appearance of isolates of strain I on 2% malt agar is typical of this species, whereas isolates of strain II are more restricted in growth and perithecial production is limited by comparison

TABLE 1. Numbers of isolates of fungi and of *Chaetomium* spp. obtained from soil samples collected from upland (P2) and marshland (R3) permanent pasture in the period 1966–1975

	Soil samples collected		Fungal cultures isolated		Isolates of *Chaetomium* spp.		Isolates of *C. umbonatum*	
Year	P2	R3	P2	R3	P2	R3	P2	R3
1966	20	4	490	55	3	2	1	0
1967	319	205	2668	2664	20	6	14	1
1968	124	116	1484	1095	8	3	8	2
1969	112	112	1292	1209	5	4	5	4
1970	116	NA	1399		8		8	
1971	48	41	570	414	3	6	3	6
1973	326	NA	4354		14		13	
1974	500	NA	6168		10		9	
1975	520	NA	7553		10		9	
	2085	478	25978	5437	81	21	70	13

NOTE: NA = no collections made.

TABLE 2. Antibiotic activity and metabolite production by *Chaetomium* spp. isolated from soils of permanent pasture at Nappan, Nova Scotia

Species	Accession number (HLX)	Date of isolation	Soil type	Growth inhibition F	Growth inhibition E	Metabolites identified	Yield, µg/ml
C. cochliodes	832	14/9/66	R3	–	+	Chetomin	0.02
" "	833	21/7/66	P2	+	+	Chetomin*	140
" "	834	2/10/67	P2	+	+	Chetomin*	7
C. funicolum I	835	10/10/67	P2	+	–		
C. funicolum II	704	21/9/67	P2	–	+	Chetomin*	0.06
C. globosum	836	10/10/67	P2	+	+	Chetomin*	2
" "	837	10/10/67	R3	+	+	Chetomin	0.01
" "	840	18/4/68	R3	+	+	Chaetoglobosin	49
" "	870	15/8/68	R3	+	+	Chaetoglobosin	3
" "	1367	9/9/75	P2	+	+	Chaetoglobosin	10
C. umbonatum	809	15/5/67	P2	+	+	Chetomin	0.3
" "	821	17/7/67	P2	–	+		
" "	822	24/7/67	P2	–	+		
" "	825	24/7/68	P2	+	–		
" "	843	8/5/68	P2	–	+		
" "	867	11/6/68	R3	+	+	Yellow pigments	
" "	875	20/8/69	P2	–	+	Chetomin	
" "	911	29/5/70	P2	–	+	Yellow pigments	
" "	940	25/6/70	P2	–	+	Yellow pigments	
" "	971	6/8/70	P2	–	+	Chetomin	
" "	972	13/8/70	P2	+	–	Yellow pigments	

NOTE: F = culture filtrate; E = extract; yellow pigments refer to the fluorescent compounds mentioned in the text; growth inhibition refers to *Bacillus subtilis* as the test organism; chetomin* refers to isolated and fully characterized metabolite; other references to chetomin refer to material having the same R_f as chetomin on TLC (Rahman *et al.* 1970); where yields have been given, they have been determined by titration (Brewer and Taylor 1967).

to strain I. The yellow-brown coloration imparted to the medium by isolates of strain II gives these cultures a distinctive appearance thus allowing this strain to be readily identified on dilution plates. Five isolations of *C. funicolum* II, one from a marshland soil sample collected in 1967 and the remainder from upland soil samples (1967 (2), 1973 (1), and 1974 (1)), were obtained and three isolations of *C. funicolum* I were found in upland soil samples collected in 1967. It is clear that species

other than *C. umbonatum* have been uncommon in recent years, possibly because these pastures have not been grazed by cattle since 1966.

Of the 102 isolates of *Chaetomium* spp. obtained, 56 have been grown in the laboratory under the fermentation conditions used for the production of chetomin by *C. cochliodes*. The results of examinations of culture filtrates and extracts, that inhibited bacterial growth, are given in Table 2. All of the isolates of *C. umbonatum* and one isolate of *C. funicolum* II lost their ability to produce antibiotics after two or three subcultivations in the laboratory. Four isolates of *C. umbonatum* produced appreciable quantities (1 μg/ml) of pigments having an intense yellow fluorescence. These pigments were detected in trace amounts in most fermentations of isolates of this species and it is possible that they are characteristic. The mutability of *C. umbonatum* isolates so far as their production of antibacterial metabolites in laboratory culture is concerned recalls the behaviour of wild isolates of *Pithomyces chartarum* (Dingley *et al.* 1962).

By contrast, isolates of *C. cochliodes* and *C. globosum* were relatively stable producers of mycotoxins in laboratory culture. Three isolates of *C. globosum* produced at least two yellow pigments that inhibited the growth of *B. subtilis*. Fermentations on the 20-ℓ scale of one of these isolates (HLX 840) resulted in the isolation of two pigments, one in about 200 times the yield of the other. The metabolite obtained in greater yield was crystalline, mp 150–156°C, $[\alpha]_D^{23}$ −307° (*c*, 0.65, MeOH) and on mass spectroscopy gave a molecular ion *m/e* 528.2612 corresponding to $C_{32}H_{36}N_2O_5$ (requires 528.2624), an elemental composition supported by analysis (Found: C 70.7, H 7.1, N 4.95, O 17.0. $C_{32}H_{36}N_2O_5$, H_2O requires C 70.3, H 6.95, N 5.1, O 17.6). Its ultraviolet spectrum λ_{max} (MeOH) 273, 280, 290 nm (log ε 3.86, 3.84, 3.73) suggested the presence of an indole, also supported by a positive Erhlich reaction and an intense peak in the mass spectrum at *m/e* 131. This data was consistent with the proposal that the metabolite was related to the chaetoglobosins (VI) (Sekita *et al.* 1973). The mass spectrum of the metabolite from HLX 840 and chaetoglobosin A (from Dr. S. Natori, Japan) were identical. We were also unable to separate the two specimens chromatographically. Comparisons of their infrared spectra, obtained in Halifax and in Tokyo, however, showed that the two compounds were different. The data available at the moment suggests that the major metabolite from HLX 840 is a new chaetoglobosin, or possibly a hydrate of chaetoglobosin A (Silverton *et al.* 1978).

Our Japanese colleagues have reported (Saito *et al.* 1971; Umeda *et al.* 1974) that their isolates of *C. globosum*, and even their cultures of *C. cochliodes* produced only chaetoglobosins and no chetomins were detected. In our collection, we have not found an isolate of *C. cochliodes* that produces a chaetoglobosin, but isolates of *C. globosum* produced both types of tryptophan metabolite (V and VI).

Acknowledgements

We thank Dr. S. Natori for samples of chaetoglobosins, and for comparing our material with his compounds; Mr. T. M. MacIntyre for facilities at the Experimental Farm at Nappan, and Mr. D. Tappen for technical assistance.

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